THE SPERMATOGENESIS OF PANDARUS SINUATUS SAY.

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The spermatogenesis of the parasitic copepods of the Woods Hole region was included as a preliminary note in a previous paper of mine (McClendon, '06), *Læmargus muricatus* Kröyer being taken as an example. The spermatogenesis of these forms is more nearly identical than the oögenesis. In fact, the greatest difference is in the number and size of the cells in the testes. The advantage in studying *Læmargus* was the greater size and greater number of cells in the testes, but the difficulty of obtaining living material of this genus led to the substitution of *Pandarus*.

The material for the present paper was procured at Woods Hole last summer. The testes with more or less adjacent tissue were removed and fixed in Flemming's stronger fluid. Paraffine sections, 3 to 7 microns in thickness, were cut, and stained in various ways. Iron hæmatoxylin, Delafield's hæmatoxylin, saffranin, orange G, and various combinations were found valuable. Not a great deal of attention was paid to the exact form in which the cytoplasm and nuclear sap was coagulated by the fixation, but attention was directed chiefly to the chromatin of the nucleus and to those remarkable bodies described in the previous paper under the name of nutritive spheres.

The spermatogonia (Fig. 1) are nearly isodiametrical cells with large spheroid nuclei. Each nucleus contains during the rest stage two or more nucleoli (plasmosomes) and a reticulum in which chromatin granules are imbedded. During mitosis sixteen chromosomes are formed and divided (Figs. 2 and 3), one half of each chromosome going to each of the two daughter cells.

The primary spermatocytes when first formed (Fig. 4) are similar to the spermatogonia except for size. The nucleoli never grow to the size they reach in the spermatogonia, probably because the prophase of mitosis begins early in the growth of the cell and the nucleoli begin to dissolve before they have had time

to grow large. The chromosomes in the early prophase (Fig. 5) are thread-like and do not show a longitudinal split as is the case in the primary oöcytes of this species. It may be that the longitudinal split is present but cannot be seen on account of the smaller size of the chromosomes, or the splitting may occur after the synapsis, at which time a division is indicated by a constriction at right angles to the plane dividing the chromosomes of each pair (see below). Another interpretation is that in the primary oöcytes the "split" represents the division between adjacent chromosomes and is therefore after the synapsis. The chromosomes are at first sixteen in number, but as they become denser some appear to be joined end to end (Fig. 6), and by this time the nucleoli have entirely dissolved. The chromosomes collect together in a dense mass so that only their ends sticking out can be distinguished separately (Fig. 7), and after the elements of this mass separate they are seen to be eight double chromosomes united end to end (Fig. 8). The chromosomes now shorten, at the same time becoming thicker (Fig. 9), and soon a second constriction transforms each double chromosome into a tetrad (Fig. 10). Each tetrad continues to shorten until the width is as great as the length (Fig. 11). The nuclear wall dissolves and the spindle is now formed (Figs. 12, 13, 14). In the equatorial plate seven tetrads are arranged in a circle and the eighth lies in the center. It is impossible to observe whether the division is reducing or not, owing to the shape of the tetrads.

The second spermatocytic division follows immediately after the first. Each of the eight diads is divided equally between the two daughter cells (Figs. 15, 16). In the spermatids thus formed the chromosomes swell up and fuse to form nuclei, and the spermatid (Fig. 18) resembles the primary spermatocyte save for the reduction in the size and the absence of nucleoli.

By the methods used no distinction could be made out between the spermatids when first formed, but they develop into structures that show as great differences as exist between the products of the testes of any species of animals that has come to my attention. Many spermatids degenerate and appear to be absorbed as food by those remaining. Some of them elongate (Figs. 19 and 20) and begin to develop into spermatozoa. Whereas the spermatids

when first formed are in groups of fours, as they elongate they collect into larger groups. The cell boundary becomes granular (Fig. 21) and the cytoplasm of adjacent spermatids fuses. Only part of this cytoplasm goes into the formation of the spermatozoa and the remainder forms a mass in the center of the group. When the spermatozoön is fully formed no distinction can be made between the cytoplasmic and nuclear parts of it in fixed preparations (Fig. 22), but it appears as a homogeneous chromatic thread tapering at each end. This spermatozoön is similar to those of barnacles and some other crustacea, and is non-motile in sea water, though it is supposed, like other crustacean spermatozoa to be stimulated to locomotion by the fluid in the ducts of the receptaculum semenis.

In some spermatids the chromatin collects into apparently homogeneous masses close to the nuclear membrane, and the nucleus grows at the expense of the cytoplasm (Fig. 23). This process continues until the cytoplasm is represented by merely a thin granular layer surrounding the distended nucleus (Fig. 24) and finally disappears entirely (Fig. 25). The nuclear sap, which is at first a thin fluid gradually becomes denser until it appears homogeneous on fixation and takes plasma stains. The time at which it acquires this power of taking stains is not sharply marked off, as it appears gradually and as it is determined by the duration of the staining and destaining process, but after all the cytoplasm has disappeared the interior of the "nucleus" is easily stained. Soon the nuclear membrane disappears and the chromatin remains adhering to the surface of the sphere of material that filled cavity of the nucleus (Fig. 26), and which was designated by the name nutritive-sphere in my former paper. The spermatozoa are often arranged with one end against one of these spheres, in a mammer similar to that in which the spermatozoa of many animals are related to the nurse cells.

When the products of the testes pass through the vasa deferentia and enter the spermatophores, the nutritive spheres form a layer next to the wall of the spermatophore, and the chromatin, which had separated as globules, forms a layer inside of the layer of spheres. Lastly the spermatozoa arrange themselves more or less radially, that is, with their ends abutting against the layer of

chromatin globules. Some chromatin globules are found in the spaces between the spheres and become pressed out of shape by a pressure that transforms the nutritive spheres into polyhedrons. In spermatophores which are attached to the female, the substance of this nutritive layer is often found to have disappeared, leaving a structure resembling thin evacuated cell walls.

It would be interesting to know the chemical composition of the nutritive spheres. Heider ('79) probably supposed them to be mucilaginous, as he called them "austreibestoff." In some mosses there is found a mucilaginous substance in the antheridia, which swells in the presence of water and forces the spermatozoa out. A similar function was attributed to these spheres in Copepods by Heider, but I have found no evidence that such is the case. When the spermatophores are attached to the female, direct communication is formed with the receptaculum semenis, and there is no reason to believe that the spermatozoa could not enter the receptaculum by their own efforts. If there is a secretion in the receptaculum that would stimulate the spermatozoa into movement, it would diffuse into the spermatophore and be effective there. I have tried staining reactions on these spheres but can say only that they have a less affinity for plasma stains than the yolk spherules of most eggs, and that they are not of a fatty nature.

The position of the spermatozoa in relation to them and the fact that their substance disappears finally, led me to assume that they furnished nourishment for the spermatozoa. It has long been held that the nucleus influenced the assimilation in the cell, but so far as I know, the idea that the nucleus could form a store-house for the nourishment of other cells is new. I hesitated in putting forward such a view in my previous paper, hoping to discover a cytoplasmic origin of these spheres, but such seems not to be the case. In *Peripatus* the spermatozoa appear to draw nourishment from the nuclei of degenerated spermatids, but it is probable that such is the case in any testis in which cell degeneration occurs. In *Pelomyxa*, Goldschmidt ('06) describes the formation of "Glanzkörper" of the plasmosomes extruded from the nucleus. These "Glanzkörper" are supposed to be glycogen. In the method by which my sections were prepared, glycogen, if present, would probably be entirely washed out.

In the insect, *Ulula hyalina* (McClendon, '02), some of the eggs become modified to serve as protection to the other eggs. These abortive eggs or "repagula" develop in different ovarian tubules from the other eggs, and may receive different nourishment, but are of interest as modified sexual elements.

For comparison, each bundle of spermatids in *Pandarus* may be said to be provided with a cytoplasmic cytophore in the middle of the bundle and a greatly modified unicellular cytophore at the end of the bundle. Cytophores and basal cells are differentiated (visibly) after the second spermatocytic division (Korscheldt & Heider) in invertebrates. Nurse cells in ovaries are differentiated at an earlier stage (Marshall, '07).

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Explanation of Plate I.

All the figures were drawn with Abbe Camera, Zeiss apochromat objective 2 mm., compensating ocular 12. They represent optical sections of varying thickness, of elements of the testes of *Pandarus sinuatus* Say.

Fig. 1. Spermatogonium. The black spheres are nucleoli.

Fig. 2. Anaphase of the last spermatogonial mitosis, seven of the divided chromosomes are shown.

Fig. 3. Telophase of the last spermatogonial mitosis.

Fig. 4. Resting stage of primary spermatocyte. The two black spheres are nucleoli.

Fig. 5. Presynapsis stage of the primary spermatocyte. The chromosomes are in the form of threads and are sixteen in number, but not all are shown in the figure.

Fig. 6. Commencement of synapsis. The chromosomes are denser than in the preceding figure.

Fig. 7. Synapsis stage. The chromosomes are so close together that they cannot be counted.

Fig. 8. Post-synapsis stage. The chromosomes have paired to form eight bivalent elements.

Fig. 9. Prophase of first spermatocytic mitosis. The chromosomes have shortened.

Fig. 10. Later prophase. The bivalent chromosomes are transformed into tetrads by a longitudinal furrow.

Fig. 11. Late prophase. The tetrads have become still more shortened.

Fig. 12. Metaphase of first spermatocytic mitosis.

Fig. 13. Equatorial plate of first spermatocytic mitosis. The eight tetrads are shown.

Fig. 14. Telophase of first spermatocytic mitosis.

Fig. 15. Metaphase of second spermatocytic mitosis.

Fig. 16. Telophase of second spermatocytic mitosis.

Fig. 17. Late telophase of same.

Fig. 18. Spermatid.

Figs. 19–22. Stages in elongation of the spermatid to form the spermatozoön.

In Fig. 21 some of the cytoplasm is being lost and the cell boundary is granular.

In Fig. 22, which represents the spermatozoön, the elongated nucleus and its thin covering of cytoplasm cannot be separately distinguished.

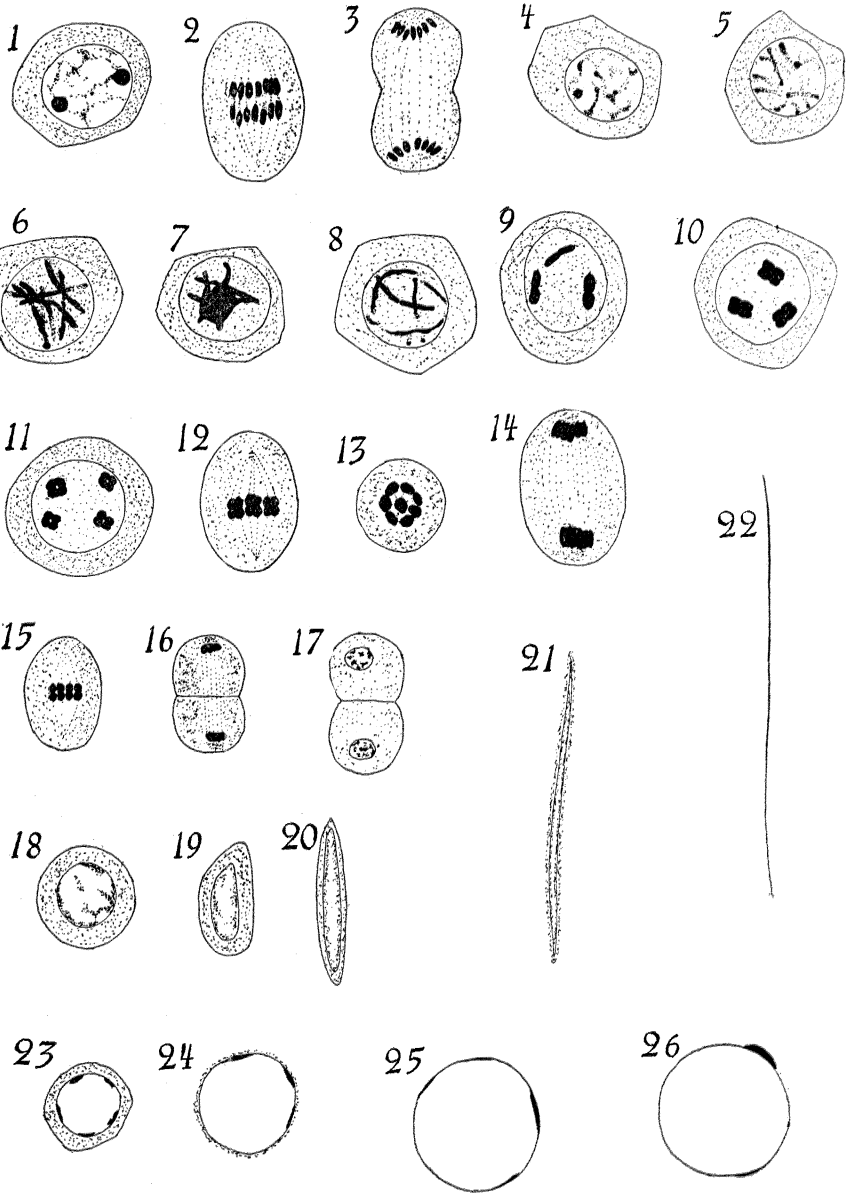
Figs. 23–26 represent stages in the formation of a nutritive sphere from a spermatid.

In Fig. 23 the cytoplasm has decreased in amount and the chromatin has collected into lumps at the periphery of the nucleus.

In Fig. 24 the cytoplasm has disappeared save for a thin granular layer, and the nucleus is distended by the nutritive sphere.

In Fig. 25 the nutritive sphere has increased in size and become denser, while the cytoplasm has entirely disappeared.

In Fig. 26 the nuclear wall has disappeared and the chromatin forms lumps on the surface of the nutritive sphere.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